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PLASMA KINETICS OF EXOGENOUS BOVINE PARATHYROID HORMONE IN CALVES

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Plasma Kinetics of Exogenous Bovine Parathyroid Hormone in Calves

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Summary. The metabolism of purified bovine parathyroid hormone-(1–84) [bPTH-(1–84)] administered i.v. by constant infusions or rapid injections was investigated in 11 normocalcemic calves. Plasma kinetics of bPTH-(1–84) were derived with a radioimmunoassay system which recognised the intact hormone but no fragments. The metabolic clearance rates ranged from 9.1–22.9 ml/min/kg. The disappearance of the intact hormone and the reversible appearance of its NH₂- and COOH-terminal fragments was investigated with additional and specific radioimmunoassay systems. At 2 min after the i.v. injection of bPTH-(1–84) a NH₂-terminal fragment (M ~ 3500) was recognised and at 4 and 20 min COOH-terminal fragments (M ~ 7000 and 3500, respectively) were detected. The survival time in the circulation of the intact hormone and of its fragments was comparable. In conclusion, bovine bPTH-(1–84) and its NH₂- and COOH-terminal fragments are metabolised in normocalcemic calves within minutes.

Key words: Half lives of disappearance – Metabolic clearance rate – Parathyroid hormone fragments.

INTRODUCTION

Bovine parathyroid hormone (bPTH), a single chain polypeptide hormone with 84 amino acid residues was isolated from parathyroid glands [2, 27]. Its amino acid sequence was identified [6, 25]. A NH₂-terminal fragment bPTH-(1–34) was synthesized and retained practically all the biological potency of the intact hormone bPTH-(1–84) [26], and a presumably biologically inactive COOH-terminal fragment bPTH-(53–84) was obtained by tryptic digestion of purified bPTH-(1–84) [25, 31]. In addition to the intact PTH-(1–84),

PTH fragments have been extracted from bovine parathyroid glands and human parathyroid tumors [12, 23, 28, 34]. Berson and Yalow [3] demonstrated for the first time that plasma immunoreactive PTH concentrations referred to the same extract as standard and also the rate of disappearance of endogenous PTH after parathyroidectomy varied when different antisera were used. The presence of several circulating PTH species with different half-lives ($t_{1/2}$) of disappearance after parathyroidectomy was subsequently demonstrated [8]. Habener et al. [16] detected only intact PTH-(1–84) in the venous drainage of bovine parathyroid glands and human parathyroid tumors, whereas Flueck et al. [14] furthermore noted considerable amounts of presumably biologically inactive COOH-terminal fragments [31] and small quantities of NH₂-terminal fragments in the venous effluent of human parathyroid tumors. Intact PTH and its fragments were estimated in higher concentrations in the venous effluent as compared to the peripheral circulation, suggesting that, in addition to intact PTH-(1–84), fragments may be secreted by the parathyroid glands. Nevertheless, bPTH-(1–84), appears to be the most important biologically active PTH form secreted in vivo by the parathyroid glands [14, 16].

When administered i.v. in vivo, bPTH-(1–84) is metabolized within minutes into NH₂- and COOH-terminal fragments [17, 18, 32]. This conversion takes place in peripheral organs such as the liver and the kidneys [9, 10, 19, 35]. Indeed, Canterbury et al. [9] recently demonstrated, by making use of the perfused isolated liver of the rat, that bPTH-(1–84) was metabolized into biologically active NH₂-terminal and biologically inactive COOH-terminal fragments. The $t_{1/2}$ of disappearance of purified non-labeled or radioiodinated bPTH-(1–84) administered i.v. have previously been estimated in several studies in human subjects, rats, calves, dogs and chickens [18, 20–22, 24, 32, 33, 35]. They were found to be prolonged in nephrectom-

ised patients, dogs and rats as compared to controls, thus pointing to the kidney as a catabolic site of the hormone [18,20,22]. The metabolic clearance rate (MCR) of bPTH based on PTH blood measurements which include only the intact bPTH-(1–84) has never been reported before.

In the present investigation the metabolism of exogenously administered bPTH-(1–84) was studied in calves. The half-lives of disappearance ($t_{1/2}$) and MCR of bPTH-(1–84) were calculated and the contribution of NH_2 - and of COOH -terminal fragments to the overall metabolism of the hormone was assessed. The in vitro degradation of bPTH-(1–84) by bovine plasma was estimated. Furthermore, the acute effects of bPTH-(1–84) on plasma calcium levels were determined in all cases.

MATERIAL AND METHODS

Peptides. A urea-trichloroacetic acid extract of bovine parathyroid glands [bPTH-(TCA)] [28] was prepared by Dr. A. Jöhl, Ciba-Geigy, Basle, Switzerland. Purified bPTH-(1–84) (lot 154 051) was purchased from Wilson Laboratories, Chicago, Ill. The preparation of bPTH-(TCA) was calculated to have a purity of 15% and purified bPTH-(1–84) of 175%, respectively, on the basis of the radioimmunological comparison with bPTH isohormone I standard 71/324 (Medical Research Council, London, England) whose purity was stated to amount to 2000 immunological units/mg. The hormonal fragment bPTH-(53–84) was prepared by tryptic digestion of purified bPTH-(1–84) [25] and donated by Dr. H. Keutmann, Boston, Mass. Synthetic bPTH-(1–34) (lot A0824) was obtained from Beckmann Instruments, Inc., Spinco Div., Palo Alto, Calif.; the purity was stated to be 5700 I.U./mg based on in vitro activation of adenylyl cyclase activity in the rat renal cortex.

Gel Filtration of Peptides or Plasma. Columns of Bio-Gel P-30 and P-10 (100–200 mesh) (Bio-Rad Laboratories, Richmond, Calif.), 1×100 cm, were used for the purification of [^{131}I]bPTH-(1–84) and -(1–34), respectively and columns of Bio-Gel P-10 (200–400 mesh), 1 and 2.5×100 cm, were used for the gel filtration of plasma samples. The columns were equilibrated with eluant consisting of 0.2 M ammonium acetate (pH 4.7) and bovine serum albumin (Sigma Chemical Company, St. Louis, Mo.) (0.5 mg/ml) at 4°C . The void volume was determined with blue dextran (Pharmacia Fine Chemicals, Uppsala, Sweden) and the salt volume with Na^{131}I . For the purification of [^{131}I]bPTH-(1–84) and -(1–34) 0.2–0.5-ml samples were used (flow rate 5–7 ml/h, 0.5–1.0-ml fractions), whereas gel filtration of plasma was accomplished with 2-ml and 10-ml samples to which [^{131}I]bPTH-(1–84), -(1–34), and Na^{131}I were added (flow rates 5–7 and 7–10 ml/h, 1.5–2.5- and 5–7-ml fractions).

The radioactivity was determined in an automatic gamma-well spectrometer (Nuclear-Chicago Corp., Des Plaines, Ill.). The amount of each labeled peptide marker was kept sufficiently low to not interfere with radioimmunological determinations.

Methods of Analysis. PTH was determined radioimmunologically according to Arnaud et al. [1] and Blum et al. [4] in peripheral plasma and in column effluent fractions with bPTH-(1–84) as standard. The following alterations were made: In addition to [^{131}I]bPTH-(1–84), [^{131}I]bPTH-(1–34) was used as ligand. Antibodies were raised in goats to bPTH-(TCA), to purified bPTH-(1–84), and to synthetic bPTH-(1–34). The hormone was estimated in lyophilised eluates from gel filtration experiments which were dissolved in radioimmunoassay (RIA) diluent. In each assay, fractions preceding the void volume were included to rule out possible non-specific effects of residual ammonium acetate on the B/F ratios. In 3–4 fractions co-eluting with the void volume the binding of trace-labeled antigen presumably to the plasma proteins was always increased, and the presence or absence of immunoreactive PTH could not be evaluated. Recoveries of immunoreactive PTH ranged from 60–110%. All samples from 1 experiment and 1 gel filtration were analyzed in the same assay. The coefficients of variation of a measurement within the same assay were up to 12% and between assays up to 15%. PTH was quantitated by means of the following systems:

A. “ NH_2 -1”-RIA System (Fig. 1A). [^{131}I]bPTH-(1–34) and anti-bPTH-(TCA) (goat 11A, day 215, diluted 1:2500). Goat 11A was immunized with 3 injections of bPTH-(TCA) totalling 2.5 mg. From the lack of inhibition by the COOH -terminal fragment bPTH-(53–84) and from the parallel slope of the inhibition curves of bPTH-(1–34) and -(1–84) it is evident that the antibodies exhibited specificities directed to determinants between amino acid residue 1 and 34. With this system PTH species other than the intact hormone were not recognised. It was therefore found suitable for the calculation of the MCR and the $t_{1/2}$ of disappearance of the intact bPTH-(1–84).

B. “ NH_2 -2”-RIA System (Fig. 1B). [^{131}I]bPTH-(1–84) and anti-bPTH-(TCA) and -bPTH-(1–34) (goat 11A, day 488, diluted 1:15000). Goat 11A was immunized with 2 additional injections of bPTH-(TCA) totalling 2.0 mg and a final injection of 0.08 mg bPTH-(1–34). The specificity of this system is comparable to the “ NH_2 -1”-RIA. However, after reimmunizations with bPTH-(1–34) affinities for bPTH-(1–34) and -(1–84) were raised 9 and 4 times, respectively. This system was found to be suitable for the detection of intact hormone as well as its NH_2 -terminal fragments after gel filtration.

C. “ COOH ”-RIA System (Fig. 1C). [^{131}I]bPTH-(1–84) and anti-bPTH-(1–84) (goat 17A, day 124, diluted 1:1500). Goat 17A was immunized with 2 injections of bPTH-(1–84) totalling 0.16 mg. From the lack of inhibition by the NH_2 -terminal fragment bPTH-(1–34), it is evident that the antibodies were directed against the COOH -terminal part of bPTH-(1–84). Considering the partial inhibition seen with bPTH-(53–84) in relation to bPTH-(1–84),

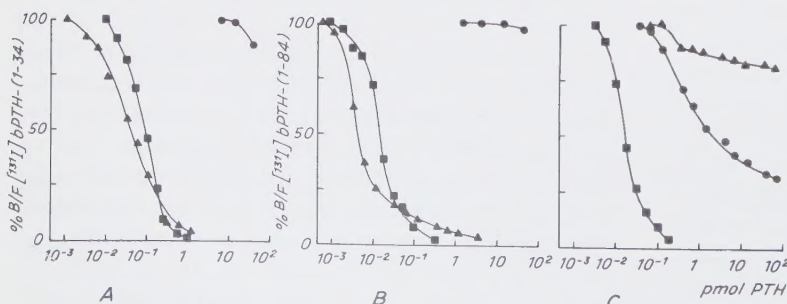


Fig. 1

Qualitative properties of antibodies to extracted bPTH used for the study of the metabolism of the hormone. (A) “ NH_2 -1”-RIA, (B) “ NH_2 -2”-RIA, (C) “ COOH ”-RIA. Ordinate: Percent initial B/F (without inhibitor added), Inhibitors: bPTH-(1–34) ($\blacktriangle$), -(53–84) ($\bullet$) and -(1–84) ($\blacksquare$) (for details see text)

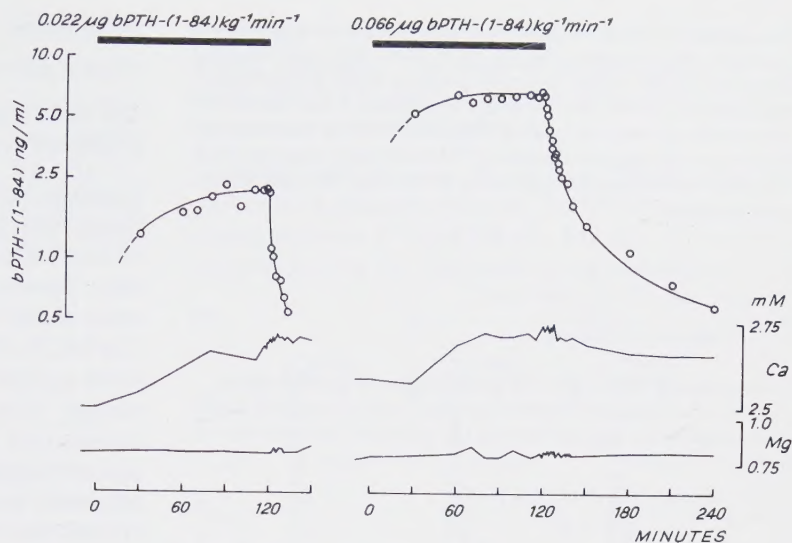


Fig. 2

Fate of bPTH-(1-84) infused for 120 min at rates of 0.022 µg/kg/min ($N = 2$) and 0.066 µg/kg/min ($N = 4$) and relationships to mean plasma calcium and magnesium levels. Plasma PTH (○) was analyzed with the “NH₂-1”-RIA system (for details see legend to Fig. 1A). Mean plasma calcium and magnesium concentrations are joined by a continuous line

affinity sites within the sequence 35–52 and 53–84 contributed to the immunological specificity. This system was used for the determination of the intact hormone and its COOH-terminal fragments in eluates from gel filtration experiments.

Plasma calcium and magnesium were determined by atomic absorption flame spectrophotometry (coefficient of variation for calcium 0.8% and for magnesium 1.3%) (Model 305; Perkin-Elmer Corp., Norwalk, Conn.). Evans-Blue (Chroma Gesellschaft, Schmid & Co., Stuttgart, Germany) was determined spectrophotometrically [30] (coefficient of variation 0.8%).

Experimental Design. Experiments were performed in 11, 2–12 week old calves with normal plasma calcium and magnesium concentrations. bPTH-(1-84) was administered as follows:

By Constant i.v. Infusions. bPTH-(1-84) (2 µg/ml bPTH-(1-84) and 1 mg/ml bovine serum albumin in 0.9% NaCl) was infused as described previously [4] into a jugular vein by means of a constant infusion pump at rates of 0.022 µg/min/kg (2 animals) and of 0.066 µg/min/kg (4 animals) for 120 min. Blood samples were taken from the contralateral jugular vein at various time intervals before, during and after termination of the infusions (Fig. 2).

By Single i.v. Injections. Five calves received 4.5 µg bPTH-(1-84)/kg (1 mg/ml dissolved in 0.01 N acetic acid) and, on the following day 2 of these calves received 0.54 mg/kg Evans-Blue (10 mg/ml in 0.9% NaCl) by rapid injection into a jugular vein and the canula was flushed with 5 ml 0.9% NaCl. Blood samples were taken from the contralateral jugular vein 3–4 times before and at various time intervals after the injections of bPTH-(1-84) (Fig. 3).

In vitro Studies. Plasma was obtained from 5 normal calves and bPTH dissolved in 0.01 N acetic acid (1 mg/ml) was added in amounts of 0.1 µg to 10 ml plasma samples kept on ice. 1 ml was rapidly frozen by immersion into liquid N₂ and the remaining plasma incubated in 1 ml portions for 2, 4, 6, 8 and 24 h at 37°C. The experiment was carried out under sterile conditions.

The plasma was collected at 4°C. Samples were stored at –20°C until use.

Numerical Analysis of Results. The analysis of the plasma PTH concentration data is based on the following mathematical model which describes the plasma concentration y as a function of the time after injection:

$$y = A e^{-\alpha t} + B e^{-\beta t} \quad (1)$$

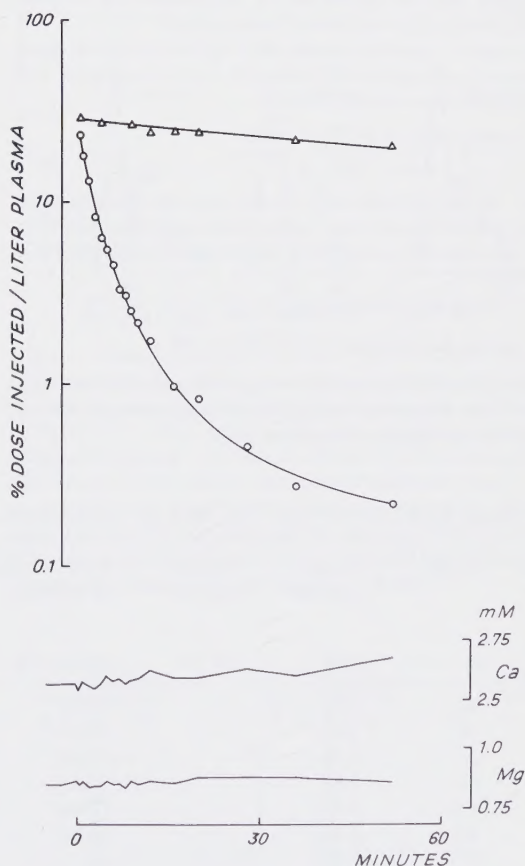


Fig. 3. Plasma disappearance of 4.5 µg/kg bPTH-(1-84) ($N = 5$) and of 0.54 mg/kg Evans-Blue ($N = 2$) administered by rapid i.v. injections and relationships to mean plasma calcium and magnesium concentrations. Plasma PTH (○) was analyzed with the “NH₂-1”-RIA system (for details see legend to Fig. 1A) and Evans-Blue (Δ) spectrophotometrically. Mean plasma calcium and magnesium concentrations are joined by a continuous line. Ordinate: Percentage of the dose injected per liter of plasma

The parameters A , B , α , β , were estimated by a least squares fit procedure. Non-weighted plasma PTH levels were used as input data to an iterative digital computer program which, if convergence was obtained, would yield parameters providing a least squares fit of estimated to observed plasma PTH levels. The fit was accepted, if the sum of the squares of plasma PTH levels (ym) minus the levels calculated (i) minus the same term of the final iteration (i) was smaller than 10^{-2} :

$$\frac{\sum_{k=1}^n (y_k m - y_k c_i)^2 - \sum_{k=1}^n (y_k m - y_k c_i)^2}{\text{degrees of freedom}} < 10^{-2} \quad (2)$$

k indicated individual and n the total number of measurements.

The initial volume of distribution (VD) was estimated by dividing the rapidly i.v. injected dose by the estimated concentration at time 0:

$$VD = \frac{\text{injected dose of bPTH-(1-84)}}{A + B} \quad (3)$$

Half lives were calculated by dividing $\log \text{nat } 2$ by the coefficient of t in the exponent of the exponential term. This estimate was based on the assumption that the rate of bPTH elimination was in every moment proportional to the plasma concentration.

In the single i.v. injection studies MCR was estimated by dividing the amount of injected bPTH-(1-84) by the calculated area under the plasma concentration curve:

$$MCR = \frac{\text{injected dose of bPTH-(1-84)}}{A/\alpha + B/\beta} \quad (4)$$

If bPTH-(1-84) was infused until a steady state was reached, MCR could be estimated directly since in the steady state the amount injected per time interval was equal to the eliminated amount. MCR was then simply:

$$MCR = \frac{\text{bPTH-(1-84) infused/min}}{\text{plasma concentration of bPTH-(1-84)}} \quad (5)$$

Plasma kinetics were calculated according to Wagner [37]. Statistical analysis was done by paired t test, double variance analysis and calculation of the coefficients of variation [36].

RESULTS

Plasma Kinetics and Acute Effects of bPTH-(1-84)

The RIA used recognized the intact hormone only ("NH₂-1" RIA).

Constant Infusions (Fig. 2, Table 1). bPTH levels were lower when 0.022 μg bPTH-(1-84)/min/kg was infused as compared to 0.066 $\mu\text{g}/\text{min}/\text{kg}$ (Fig. 2). The difference between the doses employed were significant when tested by double variance analysis ($P < 0.001$). The MCR of bPTH-(1-84) were similar when 0.022 or 0.066 $\mu\text{g}/\text{min}/\text{kg}$ bPTH-(1-84) was administered and ranged from 9.1–15.8 ml/min/kg (Table 1). After termination of the infusions plasma PTH levels decreased rapidly. $t_{1/2\alpha}$ of disappearance could only be estimated in the studies with the higher dose of bPTH-(1-84) and they varied between 3.1 and 7.5 min. As a result of the administration of bPTH, there was a rise in plasma calcium which was significant between 80 and 240 min after the start of the infusion with the higher dose ($P < 0.05$ – 0.005), whereas the plasma magnesium remained unchanged (Fig. 2). When the vehicle was infused alone, plasma calcium and magnesium levels did not rise (unpublished observations).

Single Injections (Fig. 3, Table 1). The initial volume of distribution of PTH and of Evans-Blue were in a similar range of 39.1–55.4 ml/kg. After 0.5 min the Evans-Blue levels reached a plateau, whereas PTH continued to disappear from the circulation with rapid initial half lives $t_{1/2\alpha}$ between 0.2 and 2.0 min. The second half lives $t_{1/2\beta}$ were comparable to the $t_{1/2\alpha}$ in the infusion experiments and varied between 3.3 and 20.5 min. The MCR ranged between 9.7 and 22.9 ml/

Table 1. Plasma kinetics of bPTH-(1-84) after i.v. infusions and injections

Experiment	Weight kg	Plasma calcium ^a mM	Plasma volume ^b ml/kg	Rate of infusion of bPTH-(1-84) $\mu\text{g}/\text{min}/\text{kg}$	bPTH-(1-84) injected i.v. $\mu\text{g}/\text{kg}$	VD ml/kg	MCR ml/min/kg	$t_{1/2}$	
								α min	β min
1	84	2.38	—	0.022	—	—	9.1	—	—
2	80	2.63	—	0.022	—	—	12.6	—	—
3	56	2.50	—	0.066	—	—	15.8	7.5	—
4	80	2.63	—	0.066	—	—	12.2	5.7	—
5	84	2.43	—	0.066	—	—	10.5	4.3	—
6	80	2.48	—	0.066	—	—	11.3	3.1	—
7	110	2.70	N.D.	—	4.5	55.0	16.4	0.9	5.3
8	67	2.73	N.D.	—	4.5	39.1	22.9	0.2	3.3
9	61	2.75	42.2	—	4.5	48.3	9.7	2.0	10.7
10	68	2.65	N.D.	—	4.5	45.7	15.2	0.8	5.0
11	61	2.38	55.4	—	4.5	39.7	10.5	1.5	20.5

^a Mean of 3–4 plasma calcium determinations before administration of bPTH-(1-84)

^b After i.v. injection of 0.54 mg/kg Evans-Blue

Abbreviations used: VD = initial volume of distribution; MCR = metabolic clearance rate; $t_{1/2}$ = half life of disappearance; N.D. = not done

min/kg and were comparable to the MCR in the infusion studies.

In vitro Studies. bPTH-(1–84) was incubated at 37°C in bovine plasma obtained from five calves. Mean PTH levels changed from 110.4 to 111.6 at 2 h and to 104.9 ng/ml at 24 h. The loss of immunoreactivity during the incubation was insignificantly small ($P > 0.05$).

Gel Filtration of bPTH-(1–84) and of Circulating PTH Before, During and After the i.v. Administration of bPTH-(1–84)

Bovine PTH and samples from representative i.v. constant infusion and single injection experiments of bPTH-(1–84) were subjected to gel filtration analysis and column eluate fractions were quantitated with the “NH₂-2”- and the “COOH”-RIA systems. Purified bPTH-(1–84) subsequently used in the experiments consisted of a single peak (Fig. 5). On gel filtration of plasma obtained prior to the i.v. administration of bPTH-(1–84), endogenous PTH was not detectable, whereas PTH could be quantitated in column eluate fractions obtained during and after the i.v. administration of bPTH-(1–84). As a consequence, PTH species measured on gel filtration only represent exogenously administered bPTH-(1–84) and its metabolic products.

“NH₂-2”-RIA System. Peak levels of bPTH-(1–84) were reached as early as 30 min in the constant infusion experiment (Fig. 4). NH₂-terminal fragments could not be identified.

The intact bPTH-(1–84) was discernible up to 52 min after the single i.v. injections of bPTH-(1–84) (Fig. 5). As early as 2 min after the i.v. injections a NH₂-terminal fragment was detected which co-eluted with [¹³¹I]bPTH-(1–34). This peak was followed by

a shoulder possibly representing a smaller molecular weight fragment. The rates of disappearance of the intact bPTH-(1–84) and of its NH₂-terminal fragments were comparable (Fig. 6).

“COOH”-RIA System. As early as 30 min after the start of a 2 h constant infusion of bPTH-(1–84) a peak eluting between [¹³¹I]bPTH-(1–84) and -(1–34) was recognised (Fig. 4). This latter peak corresponded to

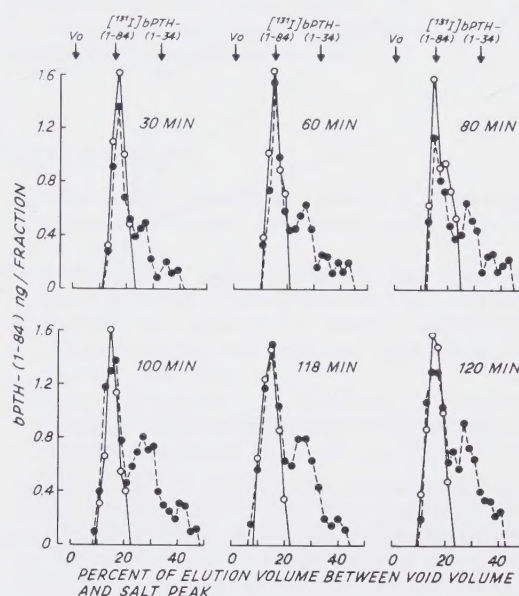
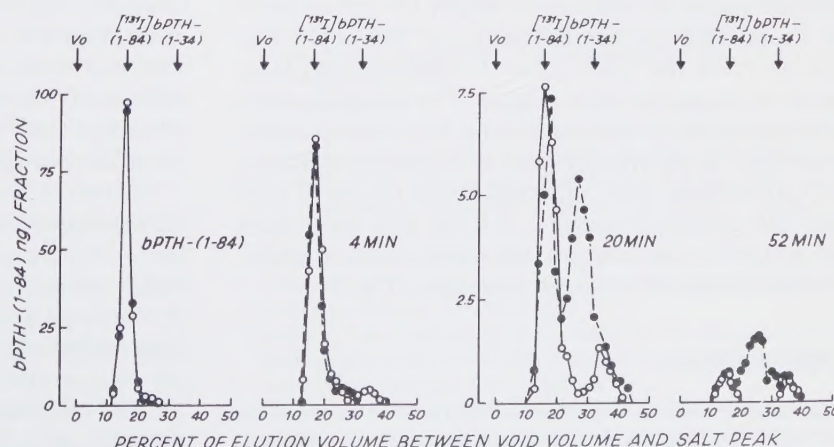


Fig. 4. Characterisation of plasma PTH at various time intervals after the start of a 2 h constant infusion of 0.066 µg/min/kg bPTH-(1–84) in calves (see Fig. 2) by chromatography on Bio-Gel P-10. [¹³¹I]bPTH-(1–84) and -(1–34) were added as marker to 2-ml samples of plasma. 1 × 100-cm column, 0.2 M ammonium acetate, pH 4.7 and bovine serum albumin 0.5 mg/ml as eluant, 5–7 ml/h flow rate, 1.5–2.5-ml fractions. The void volume (V₀) (0%) was determined with Dextran-Blue and the salt peak (100%) with Na[¹³¹I]. PTH was analyzed with the “NH₂-2” (○) and the “COOH” (●) RIA systems (for details see legend to Fig. 1B and C)

Fig. 5

Characterisation of purified bPTH-(1–84) and of plasma PTH at various time intervals after a rapid i.v. injection of 4.5 µg/kg (see Fig. 3) by chromatography on Bio-Gel P-10. 2.5 × 100-cm column, 10-ml samples, 7–10 ml/h flow rate, 5–7-ml fractions (for additional details see legend to Fig. 4). PTH was analyzed with the “NH₂-2” (○) and the “COOH” (●) RIA systems (for details see legend to Fig. 1B and C)



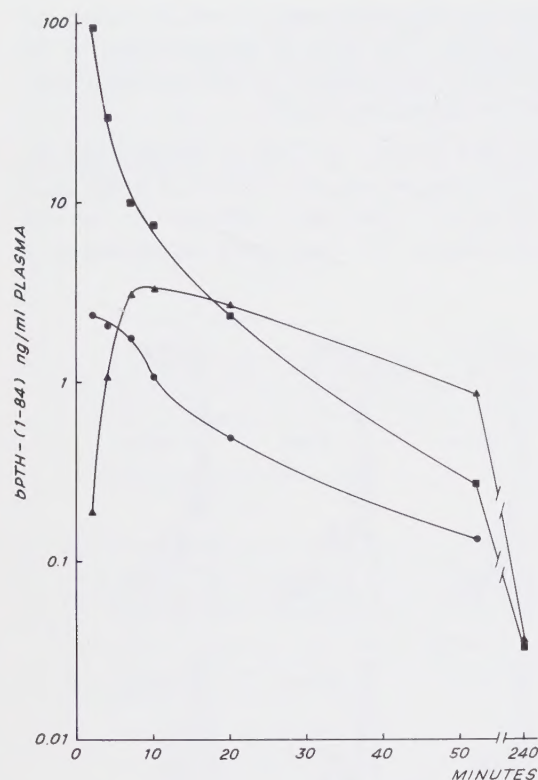


Fig. 6. Plasma disappearance of bPTH-(1-84) and reversible appearance of its fragments after rapid i.v. injections of 4.5 $\mu\text{g/kg}$ bPTH-(1-84) (for details see legend of Figs. 3 and 5). bPTH-(1-84) (■); PTH fragments analyzed with the "NH₂-2"- (●) and the "COOH"- (▲) RIA systems (for details see legend to Fig. 1 B and C)

the 7000 mol wt COOH-terminal fragment described in the literature [9, 18, 31, 34]. Already at 30 min after the start of the infusions another COOH-terminal fragment co-eluting with bPTH-(1-34) became visible. The amount of the COOH-terminal fragments remained constant between 100 min and the end of the infusions.

In the single i.v. injection experiments, a shoulder became visible in addition to bPTH-(1-84) as early as 4 min after the administration of bPTH-(1-84), and at 7 min the 7000 mol wt COOH-terminal fragment could be identified (Fig. 5). The amounts reversibly increased up to about 10 min. At 20 min a smaller molecular weight shoulder and at 52 min an additional COOH-terminal peak emerged which co-eluted with the NH₂-terminal fragment. At 240 min only traces of bPTH-(1-84) and of the 7000 mol wt COOH-terminal fragment remained detectable (Fig. 6).

DISCUSSION

The biological activity of a hormone is influenced by its rate of secretion, by its degradation in the plasma,

its elimination from the plasma compartment and its metabolism in peripheral organs, as well as by its interactions with functional sites. We undertook the present investigation with the primary aim of assessing the metabolism and concomitantly the biological response to exogenously administered bPTH-(1-84) in calves. Several studies have been performed in vivo on the $t_{1/2}$ of disappearance of exogenously administered bPTH-(1-84) [17, 18, 20-22, 24, 33, 35], but no formal analysis of the MCR of intact PTH-(1-84) has so far been reported. This appeared to us particularly important, since the major biologically active form of PTH secreted by the bovine parathyroid glands appears to be intact bPTH-(1-84), whereas in the peripheral circulation a variable amount of smaller mol wt PTH fragments contribute to the overall immunoreactivity measured with RIA systems exhibiting different specificities [8, 15, 16, 31, 34]. For this reason, we also studied the distribution of NH₂- and COOH-terminal fragments seen after the i.v. administration of bPTH-(1-84).

The present study has utilized the constant infusion to equilibrium method and rapid i.v. injections to determine the plasma kinetics of bPTH in calves. As a consequence mean rises of the plasma calcium of up to 0.21 mM occurred, which demonstrates the biological activity of bPTH-(1-84) administered. This indicates that the animals were treated with pharmacological amounts of bPTH-(1-84). However, with the lower doses infused of bPTH-(1-84) (0.022 $\mu\text{g/min/kg}$) plasma PTH concentrations reached a plateau which approximated the levels seen in cows during an acute depression of the plasma calcium concentration by only 0.30 mM induced with ethylene glycol bis (β -aminoethyl ether)-N,N'-tetraacetate (EGTA) infusions [4]. Furthermore, PTH levels attained during all the infusions of bPTH-(1-84) (with 0.022 and 0.066 $\mu\text{g/min/kg}$) never exceeded the range seen in cows with postparturient hypocalcemia [5]. MCR were similar regardless of whether bPTH-(1-84) was infused as 0.022 or 0.066 $\mu\text{g/min/kg}$ for 2 h or administered by single i.v. injections of 4.5 $\mu\text{g/kg}$. It appears that with the doses employed the metabolism was not saturated. The degradation of bPTH-(1-84) in the plasma in vitro was negligible for up to 24 h and did not affect our calculations.

Bovine PTH levels decreased immediately after termination of the bPTH-(1-84) infusions and after the i.v. injections. The results of our calculation of the initial volume of distribution of i.v. injected bPTH-(1-84) were in a similar range than the plasma volume determined with Evans-Blue which presumably binds to plasma albumin [29]. The rapid $t_{1/2\alpha}$ of 0.2-1.5 min observed after the i.v. injections but not after the constant infusion experiments probably represents

the dilution of PTH in an extracellular fluid compartment. $t_{1/2}$ of disappearance of i.v. administered bPTH of 2–9 min observed in dogs and chickens [18, 24, 35] and of endogenous bPTH during calcium infusions of 2–4 min in cows [5] are similar to our own $t_{1/2\alpha}$ results obtained in the constant i.v. infusion and the $t_{1/2\beta}$ in the i.v. injection experiments. They were more rapid than the $t_{1/2}$ of disappearance ranging from 10–30 min reported earlier with unlabeled and with radioiodinated bPTH-(1–84) in normal human subjects, rats, dogs and cows [20–22, 33], and of endogenous PTH after removal of parathyroid tumors in patients with primary hyperparathyroidism [7]. In some of these studies early timed blood samples which are a prerequisite for the characterisation of the early $t_{1/2}$ of disappearance were not taken. Alternatively, half-lives of up to 12 h have been observed in patients with normal and abnormal kidney function [3, 7, 11] after the removal of parathyroid tumors which may be caused by the slow disappearance of endogenous PTH fragments [8].

In the present study, bovine PTH-(1–84) was metabolized into NH_2 - and COOH -terminal fragments, whose reversible appearance was demonstrated on gel filtration analysis of plasma obtained during and after i.v. administration of bPTH-(1–84). The NH_2 -terminal fragment co-eluting with [^{131}I]bPTH-(1–34) probably corresponds to the biologically active 3500 mol wt NH_2 -terminal PTH and the COOH -terminal fragments to the biologically inactive 3500 and 7000 mol wt COOH -terminal forms generated by Canterbury et al. [9] by rat liver perfusions with bPTH-(1–84). After the single i.v. injections maximal levels of the 7000 mol wt COOH -terminal fragment were already reached between 5 and 20 min, whereas during the constant infusions an apparent steady state of the 7000 mol wt COOH -terminal fragment was reached at 100 min. The sensitivity of the RIA used was only sufficient for the detection of NH_2 -terminal fragments in the single i.v. injection but not in the constant i.v. infusion experiments. The times of disappearance of the COOH -terminal fragments were somewhat slower than of the intact hormone and of its NH_2 -terminal fragment. However, none of the circulating species demonstrated on the present investigation has a half-life in the range of several hours as described for endogenous PTH after the removal of parathyroid tumors from hypercalcemic patients with primary hyperparathyroidism [3, 7, 11].

The reason for the apparent different rates of the metabolic breakdown of PTH fragments in men and calves are not clear. The metabolism of purified and exogenously administered bPTH-(1–84) may not be entirely comparable to the metabolic breakdown of endogenously secreted bPTH-(1–84). The detailed

role of species differences and of changes in the extracellular calcium concentration [9, 13] on the secretion and the survival time of PTH fragments in the circulation remains to be evaluated.

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Curriculum vitae

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